# CANADIAN JOURNAL OF RESEARCH

VOLUME 25 JUNE, 1947 NUMBER 3


— SECTION D —

# ZOOLOGICAL SCIENCES

## *Contents*


Page

The California Flatheaded Borer (*Melanophila californica* Van Dyke) in Ponderosa Pine Stands of Northeastern California—*A. S. West, Jr.* - - - - - - - 97

The Proventriculus of *Staphylinus badipes* Lec. (Coleoptera: Staphylinidae)—*W. W. Judd* - - - - - - - 119

On the Chemotherapy of Caecal Coccidiosis (*Eimeria tenella*) of Chickens. III. Observations on Sulphonamides in the Intestinal Tract and Blood—*W. E. Swales and G. H. Benham* - 122



NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

## CANADIAN JOURNAL OF RESEARCH

The *Canadian Journal of Research* is issued in six sections, as follows:

| | |
|---|---|
| A. Physical Sciences | D. Zoological Sciences |
| B. Chemical Sciences | E. Medical Sciences |
| C. Botanical Sciences | F. Technology |

For the present, each of these sections is to be issued six times annually, under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada, the Royal Society of Canada, and the Chemical Institute of Canada.

Sections B and F of the *Canadian Journal of Research* have been chosen by the Chemical Institute of Canada as its medium of publication for scientific papers.



### EDITORIAL BOARD

*Representing*
NATIONAL RESEARCH COUNCIL

DR. J. B. COLLIP *(Chairman)*,
Director, Research Institute of Endocrinology,
McGill University, Montreal.

DR. PAUL E. GAGNON,
Director of the Graduate School,
Laval University, Quebec.

DR. A. R. GORDON,
Head, Department of Chemistry,
University of Toronto, Toronto.

DR. J. A. GRAY,
Professor of Physics,
Queen's University, Kingston.

*Representing*
ROYAL SOCIETY OF CANADA

DR. A. NORMAN SHAW,
Chairman, Department of Physics,
McGill University, Montreal.

DR. J. W. T. SPINKS,
Department of Chemistry,
University of Saskatchewan,
Saskatoon.

Section III

PROFESSOR J. R. DYMOND,
Director, Royal Ontario Museum of Zoology,
Toronto.

DR. H. S. JACKSON,
Head, Department of Botany,
University of Toronto, Toronto.

Section V

*Ex officio*

DR. LÉO MARION, Editor-in-Chief,
Division of Chemistry,
National Research Laboratories,
Ottawa.

*Representing*
THE CHEMICAL INSTITUTE OF CANADA

DR. R. V. V. NICHOLLS,
Associate Professor of Chemistry,
McGill University,
Montreal.

### EDITORIAL COMMITTEE

| | |
|---|---|
| Editor-in-Chief, | DR. LÉO MARION |
| Editor, Section A, | DR. A. NORMAN SHAW |
| Editor, Section B, | DR. J. W. T. SPINKS<br>DR. R. V. V. NICHOLLS |
| Editor, Section C, | DR. H. S. JACKSON |
| Editor, Section D, | PROFESSOR J. R. DYMOND |
| Editor, Section E, | DR. J. B. COLLIP |
| Editor, Section F, | DR. J. A. ANDERSON<br>DR. R. V. V. NICHOLLS<br>DR. A. NORMAN SHAW |



Manuscripts should be addressed:

*Editor-in-Chief,*
*Canadian Journal of Research,*
*National Research Council, Ottawa, Canada.*

# Canadian Journal of Research

*Issued by* THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 25, SEC. D. JUNE, 1947 NUMBER 3


## THE CALIFORNIA FLATHEADED BORER (*MELANOPHILA CALIFORNICA* VAN DYKE) IN PONDEROSA PINE STANDS OF NORTHEASTERN CALIFORNIA[1]


BY A. S. WEST, JR.[2]



### Abstract

This paper reports the findings of studies on the biology of the California flatheaded borer (*Melanophila californica* Van Dyke). Until comparatively recently this insect had been regarded as a relatively unimportant element of the western pine beetle problem. It has been shown that larval broods may spend a period of up to several years in an incipient stage in the living host tree. During this period the gallery of the incipient stage larva is healed over and the abnormal tissues thus developed interfere with normal conduction in the wood. Undetermined factors associated with the dying of the host tree initiate a fast-growing larval stage in which feeding is voracious and growth rapid.

No attempt is made to set forth the role of the species in the complex known as the western pine beetle problem. It is, however, suggested that this insect, at least in northeastern California, may play a more important role in insect caused losses in commercial stands of ponderosa and Jeffrey pine than had previously been believed. The contributions to the knowledge of the biology of the species may assist in an eventual better understanding of insect–host relationships in this particular problem.


### Foreword

The role of the numerous groups of insects attacking forest trees is in many cases complex. In particular the role of flatheaded borers (Coleoptera, Buprestidae) has been in general poorly understood. In part, an understanding of this role has been obscured by the activity of and attention paid to other agencies such as bark beetles of the genus *Dendroctonus* in relation to western pines.

During recent years considerably greater attention has been given to several buprestids, and it is to be hoped that these investigations will lead to a clearer understanding of the host relationships involved.

In general the buprestids that feed on the cambium region have been considered 'secondary' insects, that is, not capable of attacking and killing


[1] *Manuscript received in original form October 15, 1946, and as revised, March 18, 1947.*

*Contribution from the Department of Biology, Queen's University. This paper reports work completed principally during 1935-1939 when the writer was associated with the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, Division of Forest Insect Investigations. The ideas expressed herein are those of the writer and do not necessarily represent those of his associates in the Division nor in any way relate to the policy of the Division of Forest Insect Investigations as regards western forest insect problems.*



[2] *Associate Professor of Zoology, Queen's University; formerly Assistant Conservationist, United States Department of Agriculture, resigned October, 1939.*



[The April issue of Section D (Can. J. Research, D, 25 (2) : 45-95. 1947) was issued June 4, 1947.]

healthy, vigorous trees, but associated with one or more predisposing or weakening factors. Certain species can almost always be shown to be associated with trees that are decadent or dying as the result of a primary or initiating factor. The hemlock borer (*Melanophila fulvoguttata* Harr.) develops in trees (*Tsuga canadensis* (L.) Carr.) whose root systems have been wholly or partially killed by drought (Secrest *et al.*, 12) or trees whose root system has been submerged by high water (Balch, 1). *Melanophila gentilis* Lec., the pine flatheaded borer, is another example. This species develops in windfalls, slash, fire-injured trees, and trees in which the cambium has been injured by abnormally low winter temperatures. Ponderosa pine (*Pinus ponderosa* Dougl.) and Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) are the principal hosts.

Another group consists of species that cannot regularly be shown to be abundant wholly as the result of some preceding or primary factor. Such species may possibly be assigned something more than a purely passive role in the biological complex involved. Admittedly an unrecognized predisposing factor may exist. The bronze birch borer (*Agrilus anxius* Gory) furnishes one example of this type (Balch and Prebble, 2) and *Melànophila californica* Van Dyke, attacking living ponderosa and Jeffrey pines in northeastern California is another striking example.

The writer has had the opportunity of becoming thoroughly familiar with the problems posed by *M. californica* and *M. gentilis* in California and by *A. anxius* in New Brunswick and in addition has visited field study plots in Wisconsin where *M. fulvoguttata* was being investigated.

No final and complete definition of the role of *M. californica* is attempted in the following paper. However, the writer feels that the work herein reported adequately demonstrates the greater importance of the species than would be indicated by previous attention.

## Introduction

The activities of the California flatheaded borer in ponderosa pine forests of northeastern California are of considerable interest because of the association of that species in the biological complex that is commonly referred to as the western pine beetle problem. That association is particularly noticeable in the mature and over-mature commercial stands. It is more evident in the marginal stands but is present in some trees in most of the so-called east-side pine type stands in which the western pine beetle problem is important.

This western pine beetle problem exists in northeastern California because of insect activities associated with the loss of lumber values resultant from the death of merchantable ponderosa pine trees. Several species of insects may be involved, not only over given areas, but in single trees as well. The problem was given its common name more than 30 years ago because the western pine beetle (*Dendroctonus brevicomis* Lec.) had been found in most of the infested dying or dead trees on which stem analyses were made. However, other species of insects also commonly occur in infested trees. From

1931 through 1940 nearly 1700 infested trees were examined to determine what species occurred most frequently, in what portion of the tree they were found, and of what importance the infestations of the several species appeared to be. Western pine beetle broods were found in most of the trees. Maturing broods of the California flatheaded borer (*Melanophila californica* Van Dyke) occurred in 53.3% of the trees. The mountain pine beetle (*Dendroctonus monticolae* Hopk.), the red turpentine beetle (*Dendroctonus valens* Lec.), the Oregon pine engraver (*Ips oregoni* Eichh.), and the emarginate *Ips* (*Ips emarginatus* Lec.) also were involved to some extent, but infestations by those species ordinarily appeared to be of relatively minor importance in the general infestation picture.

Thus it was concluded that the western pine beetle and the California flatheaded borer were the two important entomological factors in the biological complex. It was also concluded that the flatheaded borer was of greater importance than its occurrence in slightly over half the sample would indicate since this species appeared to have successfully *initiated* the sequence of insect activity in 48.7% of all the trees. The western pine beetle followed the flatheaded borer infestations in what appeared to be supplementary, more or less secondary, attacks on many of these trees.

Since 1910 most of the research on the western pine beetle problem has been directed toward securing information on the biology, host relations, and control of *Dendroctonus brevicomis*. The accumulation of considerable valuable information has aided in the development and practice of methods to control infestations by that species.

During the same period the California flatheaded borer had been considered a species of secondary importance and little specific information had been gathered concerning it. The reappraisal of the species as an important entomological factor in the western pine beetle problem made it necessary to secure detailed information concerning its biology, host relationships, and control.

A one year life history for *M. californica* had been generally assumed, but some of the first results of this study created considerable doubt as to the accuracy of that assumption. Although Burke (5) had noted . . . . . "small, winding pitchy lines" . . . . . in connection with flatheaded borer infestations, their significance as a normal result of infestations in living trees was missed until J. M. Miller, in 1936, linked their occurrence with living *M. californica* larvae that were causing injury to the phloem, cambium, and sapwood of ponderosa and Jeffrey pines.

These preliminary observations, most of which were not in agreement with previous conceptions, emphasized the necessity of an intensive study of the species.

The objective of the work reported in this paper was to secure detailed information. When studies were initiated in 1935 considerable attention was also given to the pine flatheaded borer, *Melanophila gentilis*. It was soon indicated that this species was a minor factor in east-side pine infestations,

occurring principally in windfalls, slash, and fire-injured trees. The similarities and differences in habits of *M. californica* and *M. gentilis* have been summarized by West (15).

Since the completion of the studies reported herein some additional investigations have been carried out. These have dealt chiefly with variations in the life history. It is stressed that this paper is primarily concerned with the general and normal biology of the species and its relation to pine stands in northeastern California.

## Review of Literature

Most of the literature references to the California flatheaded borer are concerned with the taxonomy of the species or are short, general notes summarizing field observations concerning hosts, biology, and habits. In the taxonomic literature the description of the species by Van Dyke (14) and a revision of the genus *Melanophila* by Sloop (13) are of importance because of their technical description of adult characters.

Of the biological notes in the literature those of Burke (3, 4, 5) are of interest. Burke gives the geographical distribution of the species as including the states of Idaho, Oregon, and California and the hosts as ponderosa, Jeffrey, digger (*Pinus sabiniana* Dougl.), Coulter (*Pinus coulteri* D. Don.), Monterey (*Pinus radiata* D. Don.), and knobcone (*Pinus attenuata* Lemm.) pines.* Concerning the life history he mentions that pupation occurs from March to July and flight from May to August. The occurrence of larval sapwood scars in association with pitch pockets is also recorded. At that time pathologists considered that these scars were caused by lightning. Concerning the importance of the species, Burke states that ———— "it attacks and kills many second growth trees and assists bark-beetles to kill others". Keen (9, 10) added that the California flatheaded borer is very destructive to healthy, weakened, or slightly injured trees, particularly the old decadent types and those growing on rocky slopes, in fringe type stands, or other situations where soil moisture is deficient. Both Keen (10) and Graham (6, pp. 225-226) mentioned the results of some of the work reported in this paper concerning larval and attack habits of the species.

Aside from these few relatively short notes no other significant published information was found.

## Description of Developmental Stages

Six recognizably different developmental stages exist. In addition to the adult, egg, and pupal stage the larval stage can be separated into an incipient, a fast-growing, and a prepupal stage.

** Keen (10) lists Douglas-fir (Pseudotsuga taxifolia (Poir.) Britt.) and bigcone spruce (Pseudotsuga macrocarpa (Torr.) Mayr.) as reported hosts but these records could not be verified. It is probable that these two species are not normal hosts for the California flatheaded borer.*

*Adult* (Fig. 1)

The adult has a moderately convex, fairly robust form. Its dorsal surface is brownish-black with from one to three yellow maculations of variable size often occurring on each elytron. Forty per cent of 389 specimens examined had immaculate elytra. The ventral surface is a uniform greenish-bronze colour. The body length ranged from 7 to 11 mm., averaging 9.7 mm. for 231 measured females and 8.8 mm. for 238 measured males. In both sexes over 75% of the individuals measured within 1 mm. of the average length.

*Egg* (Fig. 2)

The egg is whitish when first laid, becoming yellowish with development of the embryo. It generally is flattened, oval in outline, fragile, and has a soft chorion. The average length is approximately 1 mm., the width being $\frac{2}{3}$ mm. Eggs are deposited under bark scales bordering on cracks and crevices in the bark. Although eggs may be laid singly they more often occur in groups of two to eight; the largest group observed contained 13 eggs. Dissection of 29 females revealed from 7 to 242 eggs in various stages of development. The average was 122 eggs.

*Incipient Stage* (Fig. 3)

The small larvae of *M. californica* mine in the cambium region of green trees and stimulate the development of abnormal tissues in the sapwood and phloem. This phase of larval growth, which may last for several years, has been called the *incipient stage* since it represents a distinct stage preceding later stages of larval growth and successful development to adult. It usually represents an extended period of slow or practically arrested development that often terminates in death of the individual larva or of partial or entire broods.

Incipient stage larvae range in size from less than 1 mm. to 5 or 6 mm. in length. Small larvae, recently emerged from eggs, are difficult to see even after they have mined inward through the intervening bark and phloem zones to reach the cambial region. The whitish, nearly transparent, body scarcely is visible against the similarly coloured background of phloem and sapwood tissues. Sclerotized mouth parts and the fine brownish line of injured tissues behind the larvae are useful in determining their location. In very young incipient stage larvae the first thoracic segment is not noticeably wider than the following segments. During the period spent in the incipient stage the typical club-shaped form of *Melanophila* larvae develops.

*Fast-growing Larval Stage* (Fig. 4)

The significant contrast between the incipient and fast-growing stages lies in the relatively inconspicuous feeding and slow growth of the former as compared with the voracious feeding and rapid growth of the latter. Initiation of the fast-growing stage is characterized by a sudden widening of the incipient mine (Fig. 7) and the formation of winding galleries 10 to 15 mm. in width in the phloem. The frass in such galleries is usually packed in crescent shaped concentric units (Fig. 9).

The body length of fast-growing larvae ranges from 5 to 6 mm. for larvae that have recently entered the stage to 25 or 26 mm. for larvae that are ready to enter the prepupal stage.

*Prepupal Stage* (Figs. 5 and 6)

Towards the end of the fast-growing stage the larva mines outward into the cork-like layers of bark and constructs a pupal cell. In thin-barked trees the larva may construct this pupal cell in the sapwood. During the following prepupal stage the larva lies inactive in the pupal cell with its posterior abdominal segments bent forward to lie beside the thoracic segments in a U-shaped position. The prepupal larvae is relatively short, plump, and creamier in colour as compared with the fast-growing larva.

*Pupal Stage* (Fig. 6)

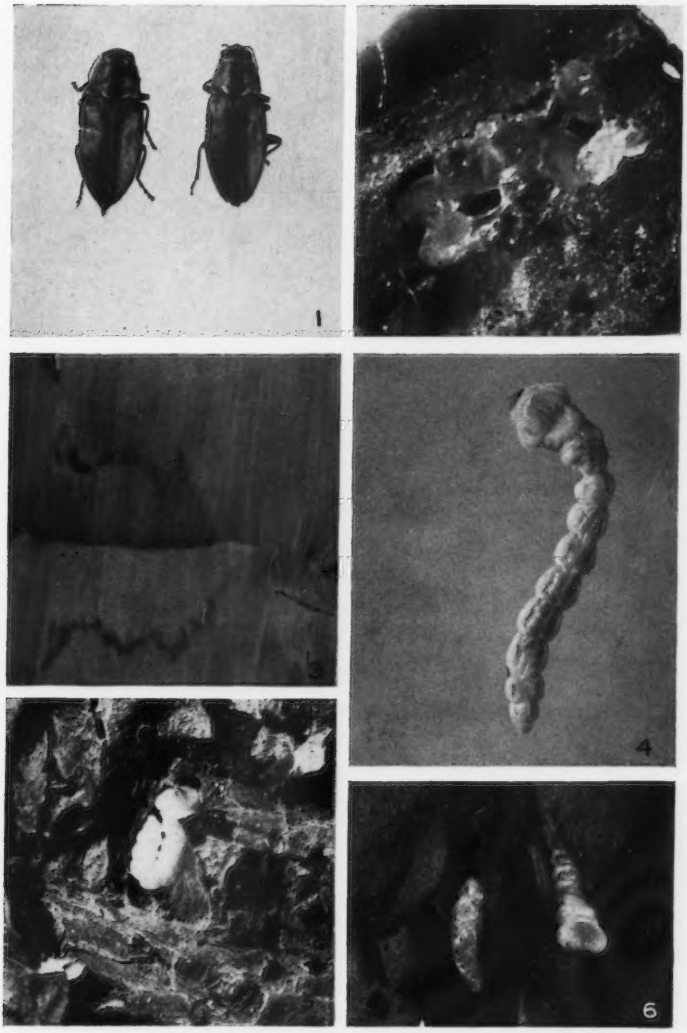
The pupa is of the exarate type with legs and wing pads folded against the body. In a majority of cases the pupa lies with its head directed toward the outer bark surface but individuals have been frequently observed in other positions. The average length of a series of pupae was about 9 mm. and the widest part of the body, in the abdominal region, was about 4 mm.

## General Life History

Disregarding minor variations that are known to exist, the life history of a given brood of *M. californica* in northeastern California proceeds about as follows.

Eggs are laid under bark scales of host trees from June through August. They hatch in from 9 to 19 days, the average length of incubation being 13.3 days. After eclosion the young larvae tunnel diagonally through the intervening bark and phloem tissues to the cambium region where they remain for an indefinite period of time feeding on the living tissues of the tree and growing very slowly. Many individual larvae never develop beyond this stage and die. Frequently death is due to 'pitching out' as a result of resistance on the part of the host tree.

In trees where further development of the brood occurs incipient stage larvae continue to feed in the cambium region until the inception of the fast-growing larval stage, which appears to be dependent on host conditions that are as yet little understood. Usually that stage occurs during June to September one, two, or three years after the eggs were laid. There is some evidence to suggest that further development may occur after as many as four years in the incipient larval stage. Ordinarily fast-growing larvae have mined out into the bark, formed a pupal cell, and reached the prepupal condition before the advent of cold weather in late September or early October. Although the prepupal stage is ordinarily reached in the fall of the year, prepupae have been observed as early as the latter part of July. The following winter is spent in the prepupal stage; pupation begins in May but some larvae may pupate as late as July. Adults start emerging in May and the peak of emergence usually occurs early in July.




FIGS. 1-6. *Developmental stages of Melanophila californica* Van Dyke.

FIG. 1. *Adult. Approx.* × *3.*

FIG. 2. *Cluster of eggs.* × *10.*

FIG. 3. *Incipient larva and galleries in sapwood and phloem.* × *2.*

FIG. 4. *Fast-growing larva.* × *2.*

FIG. 5. *Prepupal larva in characteristic position in bark.* × *2.*

FIG. 6. *Pupa and prepupal larva in bark cells.* × *2.*

FIGS. 7-9. *Larval activity.*

FIG. 7. *Initiation of fast-growing stage. Pencil on right points to start of incipient gallery. Pencil on left points to fast-growing larva and its gallery. Natural size.*

FIG. 8. *Patches of deteriorating phloem covering fast-growing larvae similar to the one shown in Fig. 7. One-half natural size.*

FIG. 9. *Half grown fast-growing larvae showing utilization of deteriorating phloem tissue and larval galleries. Approx. natural size.*

Thus the life history of the species requires two to four, and possibly five years to complete. The general life history is shown diagrammatically in Fig. 10.

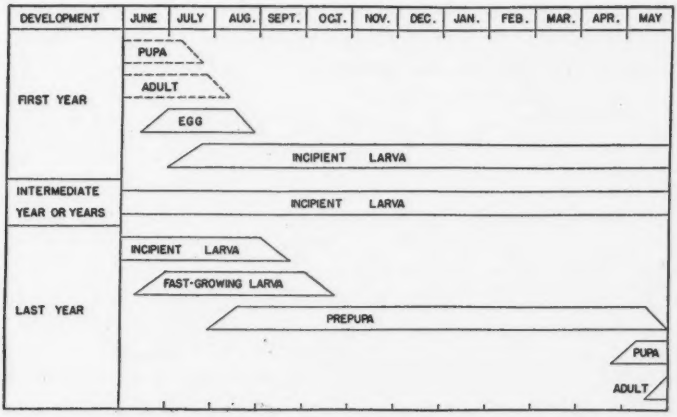



FIG. 10. *General progress of development of a single brood of M. californica.*

## Variations in the Life History

Seasonal differences affect the rate of development of single generations of flatheaded borer broods and cause minor variations in the general life history. However, the greatest source of variation results from the actions of factors involved in determining when the change from the incipient to the fast-growing stage takes place. Those factors, the exact nature of which can only be surmised at this time, appear to determine the length of period spent in the incipient larval stage, the length of time necessary to complete the life cycle of individuals and of given generations, and the number of generations represented in the populations developing in the trees.

For some of the information contained in the following paragraphs the writer has drawn on a report by Johnson (8) that dealt with *atypical* broods and host materials.

Under certain conditions the life cycle may be completed in but one year. A few trees have been encountered in which it seems certain that a part of the population emerged during the summer following the attack (Johnson (8) ). J. C. Evenden, in correspondence, reported a one year life cycle in felled poles in Idaho. In California the one year life cycle is of exceptional occurrence. Observations indicate that, in standing trees, it results from attacks made at or just before the time when conditions in the host tree become suitable for initiation of the fast-growing larval stage. Ordinarily such a condition occurs in the tree prior to the peak of adult emergence and by the time adults are ovipositing this material is no longer attractive as the result of contamination by other insect species.

In a majority of the infested trees that have been examined, three or four years have been required to complete the life cycle of individuals and of separate generations. A few individuals may, as has already been indicated, complete the life cycle in five years, which appears to be the maximum period that may be spent in developing from egg to adult. *Living incipient stage larvae have been found at the end of galleries that have been formed over a four year period* as indicated by annual rings overlying the galleries. Galleries formed over a similar four year period have been associated with broods that continued their development and emerged as adults one year after becoming fast-growing larvae.

Populations ordinarily overwinter chiefly in the incipient or the prepupal stage. However, occasionally the initiation of the fast-growing stage late in the fall season results in a relatively small proportion of the total population in the region spending the winter in that stage. In general these individuals are more susceptible to low winter temperatures than are incipients or prepupae. Ordinarily they do not continue their development in a normal manner during the following spring and most of them die before reaching the prepupal stage. A few observations have been recorded of larvae that overwintered in the fast-growing stage and subsequently completed their development successfully.

The prepupal stage is usually an overwintering phase. Exceptionally, this stage may be reached early in the spring, with pupation occurring during June or July of the same year. At the other extreme nearly a year may be spent in this stage by individuals that have been observed as prepupal larvae early in July and that did not emerge as adults until the following summer.

Numerous rearing records indicate that in practically all cases all the emergence from pieces of bark occurs during a single season. Examination of bark samples at the end of the emergence period did not indicate the presence of any diapause brood. In a few cases where *atypical* life cycle or periods of development were involved emergence from the same pieces of bark has occurred during two seasons. However, split emergence periods appear to be of infrequent occurrence.

## Development of Populations in Standing Trees

In most incipient infested trees the larval populations present are representatives of more than one generation, having resulted from attacks of more than a single season. When the life cycle of these broods continues beyond the incipient stage and is successfully completed, adults resulting from the succession of attacks emerge during a single season. Thus there commonly occurs a considerable variation in the length of life cycle of the individuals and generations of the mixed-age broods that develop successfully to the adult stage in a single tree. This results in a somewhat complicated developmental history for the aggregate population that is living and developing in the cambium region of the infested trees of a stand. A reconstruction of a representative, though not necessarily average, developmental history of such a

population is shown in Fig. 11. The explanation of this figure follows. From the time of first attack until approximately the time of initiation of the fast-growing larval period the host tree is living and usually forming annual growth rings. During the remainder of the developmental period for the flatheaded population the host material, which may be an entire tree or but a portion of a tree, is in first a dying, then a dead condition. Such feeding as takes place after initiation of the fast-growing stage is associated with the disintegration of host tissues.

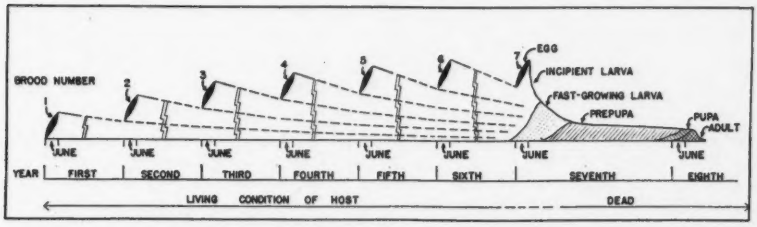



FIG. 11. *Infestation history of M. californica in standing trees.*

Successive attacks may be made in individual trees over relatively long periods of time. A detailed examination of cross sections from 35 flatheaded infested trees indicated that more than 55% of that sample had been recipient of successive attacks by incipient populations for between four and seven years. The range for the sample was from 1 to 13 years and the average between five and six years. On the basis of this information a six year period of attack has been used in Fig. 11. A seventh attack represents that of the season in which the fast-growing stage is initiated. The latter usually occurs but would not be evidenced by incipient scars in the sapwood. A year is usually required for the development from the end of the incipient stage to emergence. Thus seven and part of an eighth year may be involved in the complete developmental history of the flatheaded borer population.

Counts on approximately 130 sq. ft. of sapwood surface of infested trees showed an incipient stage larval population averaging 11.7 living larvae per square foot. A maximum population of nearly 35 living incipient larvae per square foot has been recorded. In many cases these larvae represent four seasons of attack, a point illustrated by the following table resulting from a limited series of counts made of living larvae existing in incipient infested trees during the 1937 season.

In the foregoing samples larvae resulting from the latest attacks are most abundant and those resulting from the oldest attack are least numerous. In addition to living larvae numerous scars that are not associated with living larvae occur. Often the dates of origin can be determined by cross-dating their location with the annual rings in the tree. These scars when added to the number of living larvae originating in the same year of attack provide a basis for determining the mortality of incipient larvae. The annual mortality

TABLE I

PROPORTIONS OF INCIPIENT POPULATIONS RESULTING FROM SUCCESSIVE SEASONS OF ATTACK

| Sample No. | Year of origin of living incipient stage larvae | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1934 | | 1935 | | 1936 | | 1937 | |
| | No. | % Total | No. | % Total | No. | % Total | No. | % Total |
| 1 | 0 | 0 | 3 | 7 | 9 | 21 | 30 | 76 |
| 2 | 9 | 33 | 6 | 22 | 1 | 4 | 11 | 31 |
| 3 | 0 | 0 | 12 | 25 | 15 | 31 | 21 | 44 |
| Total | 9 | 8 | 21 | 18 | 25 | 21 | 62 | 53 |

was found to be in the neighbourhood of 30%, regardless of the age of the larvae. That point is brought out in the following table giving an example of the mortality of a single season.

TABLE II

MORTALITY OF INCIPIENT STAGE LARVAE, SUMMER, 1937

| Sample No. | Year of origin of brood | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1933 | | | 1936 | | | 1937 | | |
| | No. larvae | | % Mortality | No. larvae | | % Mortality | No. larvae | | % Mortality |
| | Alive | Dead | | Alive | Dead | | Alive | Dead | |
| 1 | 3 | 3 | 50 | 9 | 4 | 30 | 30 | 1 | 3 |
| 2 | 6 | 3 | 33 | 1 | 3 | 75 | 11 | 4 | 27 |
| 3 | 12 | 3 | 20 | 15 | 6 | 29 | 21 | 20 | 49 |
| Total | 21 | 9 | 30 | 25 | 13 | 34 | 62 | 25 | 29 |

In the schematic diagram of Fig. 11 the part individuals of each year of origin play in development beyond the incipient stage has been illustrated on the basis of the foregoing data. If six successive years of attack occur in a given tree it is likely that no individuals resulting from attack periods 1 and 2 will survive until the start of the seventh year. Approximately one-third of the living larvae of each year of origin die each year. For that reason Brood 3 will have very few representatives living in the seventh year of attack.

This drain on the populations of a given year of attack has been indicated in Fig. 11 by the slope of the solid top lines and their broken extensions. The total population present in the tree at any given time is represented by the vertical distance above the base line of the diagram. Broods 4, 5, and 6 are the chief ones contributing to the population that enters and continues development through and beyond the fast-growing larval stage. Brood 7, which results from attacks at the time the remainder of the population is

entering the fast-growing stage, is the most abundant single brood represented in the population active in the tree at that time. Some individuals of Brood 7 may progress to the fast-growing stage and compete successfully with older larvae in subsequent development, but for the most part mortality in that portion of the population is usually heavy and few, if any, individuals of Brood 7 would ordinarily complete development and emerge. Thus a large portion of the mortality in the fast-growing stage, which results in a population reduction from 11.7 incipient stage larvae per square foot to about six prepupal larvae per square foot, appears to occur among individuals of the brood resulting from the seventh period of attack.

The remainder of the diagram illustrates the usual timing and sequence of events in development through the prepupal and pupal to the adult stage.

In nature the complete infestation history of individual trees is usually more complicated than is shown in this reconstructed infestation history of the California flatheaded borer. Partial infestations, such as top or strip kills, may occur, involving overlapping infestation histories. Often the initial flatheaded borer infestation in parts of a tree is followed by supplementary attacks by other species of forest insects. In this manner single or multiple infestation histories in different portions of a tree bole and the larger limbs may result and there is commonly a succession of flatheaded borer or other forest insect infestations.

A typical succession that has been commonly recognized in east-side ponderosa pine stands is illustrated in Plate III. Figs. 12*a* and 13*a* are two views of the same tree, taken over a year apart, and Figs. 12*b* and 13*b* are two diagrams illustrating the composition of infestations in the tree at the times the photographs were taken. The first photograph was taken on June 21, 1935, and the second on August 30, 1936.

Sapwood scars caused by successive infestations by incipient flatheaded borer larval broods were found along the entire bole length of this tree. Different series of incipient flatheaded borer broods reached the fast-growing stage in 1934 (in top 30 feet of bole length), and in 1935 (between 35 and 60 ft. from the base). These different series emerged as adults in 1935 and 1936 and have been labelled *FH*-1934 and *FH*-1935 in the diagrams to distinguish between them.

Supplementary attacks by adults of the 1935-1936 overwintering western pine beetle broods took place early in the spring of 1936 (*DB*-1936). The result was the pure western pine beetle infestation found from 18 to 35 ft. up the bole and the mixed flatheaded borer–western pine beetle infestation extending from 35 to 60 ft.

Another attack period, this time by western pine beetle adults of the first 1936 seasonal generation and by mountain pine beetle adults (*DM*-1936), took place some time in July, 1936. These two attacks resulted in a mixed infestation by the two species in the basal 17 ft. of the bole length.

The flatheaded borer attacks probably took place over a period of years. If we consider only that this series of successive attacks successfully completed development to adult stage in two different periods of time it is evident that four separate infestations by three species of forest insects are shown by this example.

## Habits

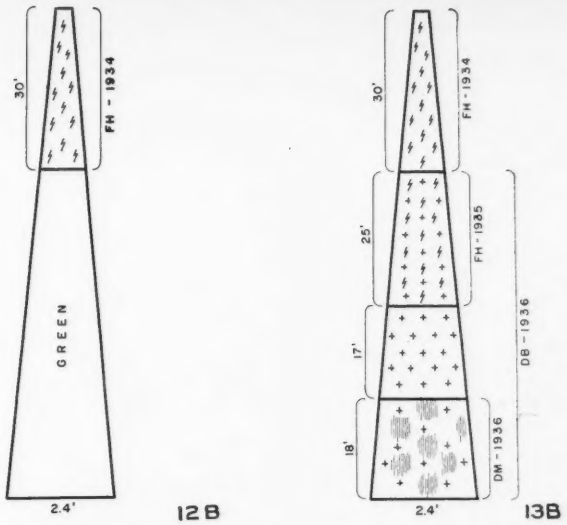
*Adult*

Adults are found in pupal cells in the outer bark of infested trees from April until late in July. The two sexes emerge at about the same time and in approximately equal numbers. The average emergence was 3.6 beetles per square foot of bark surface, from approximately 2700 sq. ft. of bark representing a number of samples over several seasons. The beetles are active on warm (70° F. and above) sunny days, but are rarely seen in flight. They can occasionally be collected from pine foliage and are attracted, often in large numbers, to windfalls, slash, and other freshly felled host material. They have been observed crawling over the bark of trees supporting heavy incipient infestations. Temperature, rather than light, appears to be the factor controlling activity. The beetles are inactive at night, in the early morning, and in the late afternoon. However, they are positively phototropic, exhibiting a preference for sunlight areas on logs and trees and moving to light-admitting apertures in darkened cages.

For a period of about 10 days following emergence, the adults spend their time flying about and feeding on pine foliage. This feeding appears to be necessary for sexual maturity. In cages neither copulation nor oviposition was observed unless fresh foliage was furnished as food for the caged populations. The effect of adult feeding under natural forest conditions is not known, although feeding injury was found on from 54 to 100% of the needles of 181 green twigs that were taken as samples from eight incipient infested trees. When relatively large populations of adults are confined in cages placed over small trees the feeding injury has been severe. This injury resulted in the death of many needles, a general thinning of the foliage and a consequent reduction of the physiologically active needle complement. The extent of injury to caged pole-size ponderosa pines is shown in Table III.

Under caged conditions the beetles preferred needles of the current or previous season although some feeding occurred on needles of all ages. Individual needles on which adults have fed exhibit a series of irregular serrations along the edge. Ordinarily the edges of the serrations are not clean cut. When the injury extends well into the centre the needle usually dies beyond the point of attack, or the entire needle may be killed.

There is considerable variation in length of adult life. Under cage conditions, with fresh pine foliage supplied as food, one adult lived for 62 days after emergence. The average length of life of 344 adults under those conditions was 13 days. Without food the average age at death was but four days, with a maximum of 15 days.



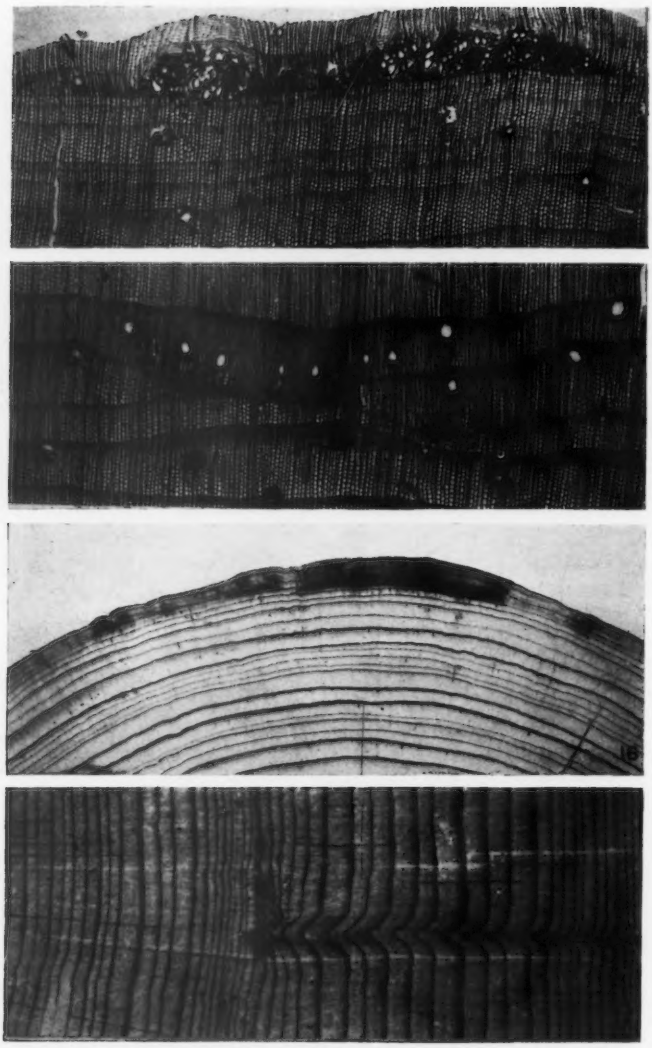

FIGS. 12 and 13. *Example of a common sequence of successful infestations in east-side ponderosa pine trees.*

FIG. 12*a*. *Picture of tree on June 21, 1935.*

FIG. 12*b*. *Diagram of infestation in tree on June 21, 1935.*

FIG. 13*a*. *Picture of tree on Aug. 30, 1936.*

FIG. 13*b*. *Diagram of infestation in tree on Aug. 30, 1936.*



FIGS. 14-17. *Effect of incipient galleries on wood tissues.*

FIG. 14. *Cross section of incipient gallery overlaid by wood tissues showing resin infiltration and parenchymatous tissue.* × *16.*

FIG. 15. *Cross section of old incipient gallery in sapwood showing growth disturbance and abnormal concentration of resin ducts.* × *25.*

FIG. 16. *Pitch pocket resulting from incipient attack. Natural size.*

FIG. 17. *Old incipient gallery and pit showing effect on subsequent growth.* × *2.*

TABLE III

EXTENT OF INJURY CAUSED BY FEEDING OF ADULTS ON NEEDLES OF CAGED PONDEROSA PINE POLES

| No adults in cage | Age of needles[1] | | | | | |
|---|---|---|---|---|---|---|
| | Current season | | One year old | | Two years old | |
| | Fascicles injured, % | Needles injured, % | Fascicles injured, % | Needles injured, % | Fascicles injured, % | Needles injured, % |
| 214 | 61.6 | 39.6 | 69.1 | 41.6 | 9.9 | 4.6 |
| 358 | 31.4 | 16.7 | 32.3 | 19.4 | 2.3 | 0.9 |
| 173 | 22.8 | 12.9 | 2.6 | 1.2 | 4.8 | 2.3 |

[1] *Ponderosa pine normally has a needle complement of three seasons while in Jeffrey pine the needles are retained for six to nine years.*

*Oviposition*

When ready to oviposit the adult crawls about over the bark and, when a suitable place is found, exerts the ovipositor to as much as two-thirds the length of the body. The eggs are placed either singly or in small groups on bark scales bordering on the crevices between bark plates. The percentage hatch of a sample of 1597 eggs observed was low, averaging but 50.8%.

*Incipient Stage*

Hatching occurs through the side of the egg and the larva mines diagonally through the bark to the cambium region. There, until the larva attains the fast-growing stage or dies without successfully completing the incipient stage, it feeds on the tissues of the cambium region.

The feeding injury consists of the excavation of a shallow mine in the cambium and immediately adjacent innermost phloem and outermost wood tissues. Technically the open mine or gallery of the incipient stage larva extends but a short distance behind the larva. Cellular proliferation heals the gallery relatively soon after the injury occurs. The gallery may extend ahead of the larva for about a quarter of an inch. The larva itself lies in an irregularly shaped shallow cavity that may be lobed as a result of the larva starting to mine in more than one direction. Frass fills the cavity immediately behind the larva.

The healed over mines have been named incipient galleries and are the result of typical reaction by the trees to wounding. These galleries vary in length from less than an inch to as much as five or six inches and represent the extent of feeding by individual incipient stage larvae during the period of time spent in that stage. In the sapwood the reactions of the tree form a ridge of tissue (Figs. 3, 7, 14) infiltrated with pitch. Proliferation of parenchymatous tissue, dislocation of wood tracheids, the production of an abnormal number of resin ducts (Figs. 14 and 15), and a lack of continuity of medullary rays through the wound tissue are usually the results of the feeding injury. Usually the growth of the season following the injury is normal but the annual

ring over the gallery often is wider or narrower than in adjacent uninjured areas (Fig. 15). Sometimes growth of a normal character or amount does not occur for several years.

When the larva dies without completing the incipient stage the point in the tree at which death of the larva occurs usually is marked in one of three ways. If the tree actively resists the attacks a flow of resin may overcome and imbed the larva. Subsequent healing leaves a pitch blister as a defect in the wood (Fig. 16). In the absence of very active resistance on the part of the tree the point of death may be marked by a pitchy fleck that subsequently becomes covered with normal wood tissue. Sometimes a pit is formed in association with the pitch fleck and distortion of annual rings of subsequent growth may involve the development of many seasons (Fig. 17).

Phloem disturbance is evidenced in brown, corky, injured cells whose pattern is a counterpart of the sapwood incipient gallery.

*Fast-growing Larval Stage*

The factors resulting in initiation of the fast-growing stage are unknown, but it appears that cessation of growth and probably death of the tree or infested portion of the tree occurs at about this time. Change in the character of the larval mine and in the rate of larval feeding and growth are unmistakable evidence of the transition from incipient to fast-growing stage. The mine is abruptly widened (Fig. 7) beyond the point where the incipient gallery ends. Frass is packed in crescent shaped units instead of being scattered loosely. Larvae feed voraciously on phloem tissue and increase in size very rapidly. When the outer bark is carefully removed from the phloem at the time of initiation of the fast-growing stage, browned phloem patches can be observed (Fig. 8). Living larvae are found under these patches. The mines are extended rapidly and feeding results in a relatively high utilization of deteriorating phloem tissue (Fig. 9).

## Brood Mortality

*Natural Enemies*

Little is known concerning the natural enemies of *M. californica*. Relatively few instances of parasitism and predatism have been encountered. In the course of studies on the egg stage a small hymenopteran was discovered parasitizing eggs immediately after they were placed. The parasite was determined by A. B. Gahan as a new species of an encyrtid of a genus not previously known to attack buprestid eggs. The percentage of parasitism was low. In a study made under conditions that did not interfere with the normal activity of the parasite, 2.9% of 1178 eggs were attacked.

A few parasitic Hymenoptera have emerged from bark samples containing flatheaded borer broods but no host–parasite relationship was specifically established. The numbers involved were so small as to indicate no appreciable effectiveness of parasites of the California flatheaded borer. The protected position of borer broods under the bark naturally limits the opportunity for parasitism to occur.

Clerid and ostomatid predators, chiefly *Thanasimus lecontei* (Wolc.) and *Temnochila virescens* (Fab.) have been observed attacking flatheaded borer larvae in infested trees. Larval stages of these predators commonly are found under the bark of infested trees after attacks by bark beetles have provided a means of access to the inner bark region.

Woodpeckers, chiefly the arctic three-toed woodpecker (*Picoides articus*) commonly destroy a considerable proportion of the overwintering prepupal larvae. In 1938 a count on infested bark taken from standing trees showed 23% of the original overwintering prepupal brood had been destroyed by woodpeckers.

*Competition*

Under some conditions competition for food appears to be an important factor in reducing flatheaded borer populations. Instances have been observed in which other forest insects, particularly the engraver beetle (*Ips oregoni*) and the western pine beetle (*Dendroctonus brevicomis*), have overwhelmed flatheaded borer broods. These insects, attacking soon after the flatheaded broods have reached the fast-growing stage, develop more rapidly than the flatheaded larvae. Most of the available food is consumed before the latter have completed their development and a large portion of the brood dies. There appears to be a considerable variation in the mortality resulting from this competition. This variation largely stems from the timing of attacks relative to the stage of development reached by the flatheaded borer broods. The earlier these attacks occur after the fast-growing stage has been reached the greater is the resulting mortality in the fast-growing larval population. If the attacks occur when the fast-growing stage is well advanced the flatheaded larvae are able to withstand competition and many of them continue their development to a successful emergence. Success in withstanding competition depends, too, on the species of competitor and its rate of development. For example, the Jeffrey pine beetle (*D. jeffreyi*) in Jeffrey pine develops more slowly than does the western pine beetle in ponderosa pine. Flatheaded borer broods compete more successfully with the Jeffrey pine beetle.

*Temperature*

The normal overwintering stages of the flatheaded borer usually withstand minimum temperatures even though such temperatures are low enough to cause considerable mortality of broods of other forest insect species overwintering in the same infested trees. The fast-growing stage seems to be most susceptible to the effects of low temperature. In laboratory tests exposure for two hours to a temperature of −15° F. resulted in mortality as high as 62.5%.

Air and the resulting bark temperatures during the spring and summer ordinarily do not constitute a mortality factor. However, some effects are evident when temperatures of 50° F. or less retard the pupation of overwintering prepupal larvae during the spring season. In broods that were

experimentally exposed to temperatures between 50° and 55° F. for periods ranging from 64 to 93 days emergence was reduced 24 to 61% as compared with brood exposed to normal temperatures.

## Nutritional Factors as Relating to Mortality and Host Materials

Experimental studies to determine in what manner the character and qualities of available host material relate to success of development of flatheaded larvae broods were not undertaken. It is already apparent that the host–insect relationship is an important one in determining if and when broods of the California flatheaded borer are to develop beyond the incipient stage and the degree of success of development beyond that stage. Presumably this relationship would be largely concerned with nutritional factors.

The rate of mortality in the incipient stage is high. Following attack, even in the least resistant trees, a period of feeding by incipient larvae is the rule. It has already been indicated (Table II) that when the incipient stage lasts through more than one season the annual mortality is in the order of 30%. Quite probably the terms 'host resistance' and 'nutrition' are synonymous as relates to the success of incipient larvae.

Apparently host resistance serves to hold back growth and development of the incipient stage larvae. The transition from incipient to fast-growing stage appears to involve changes either in the character of the food available or in the ability of the larvae to utilize that food. The success of subsequent development of broods in the fast-growing stage also appears to be conditioned by the physical and chemical nature of the food available. These assumptions have considerable basis if interpretations of the brood and emergence data for different types of host materials are possible at this time.

Although the tissues of the cambium region of living trees appear to be the normal food of incipient larvae, the relatively high rate of mortality and the apparent inability of the larvae to develop beyond that stage until some change takes place in the host tree suggests an imperfect adaptation of the insect to conditions in the living tree. On the other hand, the distinct preference for living trees for attack, the ordinarily poor development and high rate of mortality in fresh slash, and the inability to attack successfully in the uninfested cambium region of green slash suggests a somewhat similar imperfect adaptation to slash host material. In its biology this species is thus closer to the fir flatheaded borer (*Melanophila drummondi* Kby.), which Hopkins (7) considered to be an important enemy of Douglas fir or to the bronze birch borer (*A. anxius*, Balch and Prebble, 2) than to the hemlock borer (*M. fulvoguttata*), which Secrest *et al.* (12) consider to be secondary and relatively unimportant in the dying of the eastern hemlock. The pine flatheaded borer (*M. gentilis*) is another example of the latter type.

It is emphasized again that there is an evident need for experimental studies to determine how the character and qualities of the available host material affect the development of both incipient and fast-growing larvae and what are

the normal nutritional requirements of the species if there is to be an adequate factual basis for interpreting the role of the species in the complex western pine beetle problem.

## Host Materials

Broods of *M. californica* develop to maturity in the boles and larger limbs of standing trees, windthrown or otherwise injured trees, and in cull logs, tops, and the larger limbs left as slash following logging operations. It is emphasized that with rare exceptions, regardless of type of host material from which the emergence occurs, healed incipient galleries have been found thus indicating that attack occurred in the *living tree.* Successful attacks occur on fresh phloem but deteriorating or browned phloem is either not attractive or not suited to the development of the early larval stages.

Emergence of adults gives the most readily available indication of the suitability of various types of host material and makes possible a comparison of the relative success of *established* incipient broods in the various types of host material. In all cases bark for emergence samples was removed from trees or logs only after the brood had reached the prepupal or pupal stage of development.

### *Standing Trees*

The attractiveness of standing trees as normal host material is indicated by the occurrence of incipient populations of medium to heavy intensity in 29% of a random sample of 395 ponderosa pines. The condition of the host tree as a factor in selection for attack, or in success of establishment of incipients, is indicated by the higher incidence of incipient broods in so-called high-risk green trees (Salman and Bongberg, 11). However, the attractiveness of certain individuals or types of trees does not necessarily indicate their suitability for development of the broods to maturity.

Emergence from numerous samples of bark varied from zero to a maximum of 14.6 beetles per square foot of bark surface. This variation was exhibited in records of different individual trees or even different parts of a single tree. The average figure for several seasons are given in Table IV.

TABLE IV

ADULT EMERGENCE FROM BARK TAKEN FROM NATURALLY INFESTED STANDING TREES

| Season of record | Sq. ft. in bark sample | Emergence, no. beetles per sq. ft. |
|---|---|---|
| 1936 | 319 | 3.6 |
| 1937 | 305 | 2.9 |
| 1938 | 500 | 4.2 |
| 1939-1940 | 71 | 5.7 |
| All records | 1195 | 3.8 |

Strip-killed or top-killed material usually gave a less than average emergence. The same was true of bark from the lower bole region of infested trees. In 1936 a 60 sq. ft. bark sample, taken from the basal 10 ft. of five infested trees, gave an average emergence of only 1.2 beetles per square foot and emergence from all five samples was less than the seasonal average of 3.6. Similar poor emergence from the basal bole region has been noted for other insects attacking ponderosa pine.

*Slash, Cull Logs, and Tops*

Slash may result from logging operations during any month of the year. Such material may be uninfested, may contain incipient broods, or occasionally broods in infested but still green trees have reached the early fast-growing stage. Up to the time the investigations reported in this paper were completed it had not been possible to rear *M. californica* adults from slash or other cut material that was uninfested at the time of cutting. Oviposition and light early incipient infestations have been forced on previously uninfested logs in cage experiments but in no instance was the brood carried successfully to maturity. However, J. C. Evenden of the Coeur d'Alene laboratory of the Bureau of Entomology and Plant Quarantine has reported in correspondence that *M. californica* adults have been secured from previously uninfested ponderosa pine slash. Thus it may be concluded that the establishment and development of flatheaded borer broods can occur in slash after it has been laid down, but in northeastern California this type of infestation is not known to occur.

In slash from incipient infested trees the fast-growing stage is initiated soon after cutting when cutting occurs during the summer months. The stage of development at the time the slash is laid down appears to be important in determining the degree of success of broods in subsequent development to maturity, as is illustrated in the following table.

TABLE V

EMERGENCE FROM SLASH

| Year | Cut with brood in incipient stage | | Cut with brood in fast-growing stage | |
|---|---|---|---|---|
| | Sq. ft. bark in samples | Emergence per sq. ft. | Sq. ft. bark in samples | Emergence per sq. ft. |
| 1936 | 156 | 3.3 | 174 | 2.1 |
| 1937 | 55 | .5 | — | — |
| 1939-1940 | 22 | 4.6 | 143 | 6.7 |
| All samples | 233 | 2.8 | 317 | 4.2 |

Apparently slash created when the brood is in the fast-growing stage is more satisfactory host medium for subsequent development than is slash created when the brood is in the incipient stage. Analysis of individual records

indicates that the average emergence figures given in the preceding table are not entirely representative and that in general slash is not a particularly favourable type of host material.

On the whole, experience indicates that the cutting of trees containing incipient stage larva will initiate the fast-growing stage. Subsequent development appears to be variable, generally being abnormal and relatively unsuccessful. It may be assumed that much of the variation in development of broods in slash is associated with differences in nutritional factors. This assumption cannot be substantiated until more is known of the nutritional requirements of flatheaded larvae and of the physico-chemical changes that take place in host material after it is cut.

*Windfalls*

Records from 112 sq. ft. of bark samples taken from incipient infested windthrown trees gave an average emergence of only 1.7 beetles per sq. ft. It would appear that this type of host material also is an unsatisfactory medium for the development of those broods to maturity. As in the case of slash, competition by other insect species is one of the important factors regulating development of established incipient broods.

*Injured Trees*

Many trees that become infested by forest insects, including flatheaded borers, are fire-scarred or otherwise injured at the base. The question of the effect of these and other injuries has frequently been raised. In 1939 four incipient infested pole-size ponderosa pines were girdled by a saw kerf to test the effect of girdling on subsequent development of the broods. Two poles were girdled by twisting a heavy wire tightly around the base. The effect of that constriction would not be evident for several years. Two trees of the first series died in 1940. In one of these woodpeckers removed the major portion of an apparently heavy prepupal brood before samples could be taken. Adults of *M. californica* were secured from the bark of another dead tree in 1941. This tree had been girdled at a height of 6 ft. Above the girdle there was an emergence of 14.8 beetles per square foot. Below the girdle incipient development continued for a time and the fast-growing stage was reached but apparently no individuals reached the prepupal stage. In a pole that was practically girdled at the base by fire about a year before it succumbed to the flatheaded borer, emergence averaged 8.5 beetles per square foot of bark surface. No definite conclusions can be drawn from information supplied by two trees but the very successful brood development above injuries that disrupted conduction in the phloem may be significant.

## Effect of Insect on the Host

At the present time no attempt is being made to define the role of the California flatheaded borer as a factor in the western pine beetle problem. There remains to be done a considerable amount of investigation before the cause and effect phases are clear.

The occurrence and greater success of the borer in weaker or decadent types of trees has been noted. Thus a preceding factor, climatic or other, may relate to the incidence and success of attack. Regardless of the existence of the predisposing factor the habits of the species are such that a detrimental effect on the host may result. The borer may be one factor in bringing about the death of the tree. Commonly the western pine beetle is the last factor in the chain of phenomena leading to death, but some trees may die with the flatheaded borer being the only insect present.

The possible effects of defoliation by adult feeding are probably negligible. However, the occurrence of abnormal wood tissue as the result of incipient larval activity may have a significant effect. Any attempt at explanation of the relation of incipient galleries to the host tree must consider the effect of such galleries on conduction in the wood. Although admittedly a single gallery in itself would be insignificant, the sum total of galleries may present a considerable amount of abnormal tissue in the line of conduction.

Initial experiments on the relation of incipient galleries to conduction have indicated that some interference with normal conduction occurs. These experiments consisted of injecting a stain into small pieces of wood, logs, and entire trees and examining the conduction pattern in relation to the incipient galleries present. Conduction does not occur through the abnormal parenchymatous tissues that heal the larval mines. The possible physiological effects of this interference would be intensified according to the degree of infestation present in a tree. It is generally understood that the outer layers of wood are the most physiologically active. Thus a succession of incipient attacks may seriously reduce the conducting capacity of the bole.

When standing living trees were injected the stain failed to reach the top of trees that supported moderate to heavy incipient infestations. Since such trees had a poorer needle complement as compared with check trees in which the stain reached the top, the result is by no means entirely due to the presence of incipient galleries. It is of importance to note that in all cases the conduction stream broke up in the region of the bole where incipient galleries were present.

Although there is the evidence that incipient galleries interfere with normal physiological activity, considerably more study is necessary before the effect of this interference and the consequent role of the borer can be evaluated.

## Summary

The frequent occurrence of the California flatheaded borer (*Melanophila californica* Van Dyke) in infestation of commercial pine stands of northeastern California and the lack of specific information concerning its life history and habits have warranted an investigation of the species as a step in determining its place and importance in the so-called western pine beetle problem. This insect is known to infest several species of pine, but in northeastern California is important chiefly because it is concerned in insect-caused losses in valuable mature stands of ponderosa and Jeffrey pines.

The eggs are laid in bark crevices on living trees from about mid-June through August. After hatching, the larvae bore through the phloem to the cambium region where they feed on living tissues for a period varying from a few months to a maximum of about four years. In general the majority of larvae spend one, two, or three years in the cambium region in what has been termed the incipient stage. Ultimately these larvae die if the tree does not succumb, or if the tree dies they pass to a fast-growing stage in which feeding is extensive and growth is rapid. Usually fast-growing larvae are found from mid-June to mid-October but may occur at any time of the year. The prepupal larval stage first appears in the outer bark late in July, but pupation does not occur until April and May of the following year. Adults are active from late in May until early in August.

In standing trees, infestations are complicated by the occurrence of successive attacks over a period of years. Larvae of different ages that result from these successive attacks live together in the cambium region. The situation is further complicated by supplementary attacks in flatheaded borer infested trees by several bark beetle species.

Standing trees are infested in significant numbers. The older, decadent, or unhealthy trees have a higher incidence of infestation but thrifty and vigorous trees are not immune to attack. Adults have also been reared from slash or cull logs from trees infested while living and from windthrown and injured trees.

More work remains to be done, particularly on the host–insect relations before it will be possible to definitely determine the role of this species.

## Acknowledgments

The work reported herein was carried out between 1935 and 1940 in the field at Hackmore, Modoc County, Calif. and in the laboratory at Berkeley, Calif. From 1935 through 1938 the work was supervised by K. A. Salman assisted by J. W. Bongberg in 1935 and the writer in 1936, 1937, and 1938. In 1939 the writer was in charge of the work and in 1940, J. W. Johnson worked under the direction of K. A. Salman in completing a few unfinished phases of the project.

The contributions of R. F. Anderson, H. E. Burke, Jr., D. P. Furman, D. R. Rankin, and T. L. Thompson, who acted in capacity of field or laboratory assistants at various times, were of considerable importance. Grateful acknowledgment also is made to those former associates in the Division of Forest Insect Investigations of the Bureau of Entomology and Plant Quarantine who have aided the work by their criticisms and suggestions. In particular the writer is indebted to J. M. Miller, formerly in charge of the program of forest insect investigations at the Berkeley laboratory, for his advice and counsel, and to K. A. Salman, formerly Entomologist, under whose direction this study was conducted, for detailed assistance in revisions of this manuscript. Credit for photographs is due to K. A. Salman.

## References


1. BALCH, R. E. Notes on the habits of attack of the hemlock borer. Can. Entomologist, 67 : 90-92. 1935.
2. BALCH, R. E. and PREBBLE, J. S. The bronze birch borer and its relation to the dying of birch in New Brunswick forests. Forestry Chron. 16 : 179-201. 1940.
3. BURKE, H. E. Notes on some western Buprestidae. J. Econ. Entomol. 10 : 325-332. 1917.
4. BURKE, H. E. Flatheaded borers affecting forest trees in the United States. U.S. Dept. Agr. Bull. 437. 1917.
5. BURKE, H. E. Biological notes on some flatheaded barkborers of the genus *Melanophila*. J. Econ. Entomol. 12 : 105-108. 1919.
6. GRAHAM, S. A. Principles of forest entomology. 2nd ed. McGraw-Hill Book Co., Inc., New York. 1939.
7. HOPKINS, A. D. On the study of forest entomology in America. U.S. Dept. Agr. Bull. 37 (N.S.). 1902.
8. JOHNSON, J. W. Some variations in certain phases of the biology of *Melanophila californica* Van Dyke at Hackamore, Modoc County, California. Unpublished report. U.S. Dept. Agr. Bur. Entomol. Plant Quarantine. Division Forest Insect Investigations, Berkeley, California. 1941.
9. KEEN, F. P. Insect enemies of California pines and their control. Calif. Dept. Natural Resources Bull. 7. 1929.
10. KEEN, F. P. Insect enemies of western forests. U.S. Dept. Agr. Misc. Pub. 273. 1938.
11. SALMAN, K. A. and BONGBERG, J. W. Logging high-risk trees to control insects in the pine stands of northeastern California. J. Forestry, 40 : 533-539. 1942.
12. SECREST, H. C., MACALONEY, H. J., and LORENZ, R. C. Causes of the decadence of hemlock at the Menominee Indian Reservation, Wisconsin. J. Forestry, 39 : 3-12 1941.
13. SLOOP, K. D. A revision of North American buprestid beetles belonging to the genus *Melanophila*. Univ. Calif. Pub. Entomol. 7 : 1-20. 1937.
14. VAN DYKE, E. C. New species of Buprestidae from the Pacific States, No. 2. Entomol. News, 19 : 53-58. 1918.
15. WEST, A. S., JR. Biological notes on two species of *Melanophila*. J. Econ. Entomol. 34 : 43-45. 1941.

# THE PROVENTRICULUS OF *STAPHYLINUS BADIPES* LEC. (COLEOPTERA : STAPHYLINIDAE)[1]

By W. W. Judd[2]

## Abstract

The proventriculus of the rove-beetle, *Staphylinus badipes* Lec., is described. It is similar in form to the proventriculi of beetles of the Staphylinidae and Silphidae, described by other authors, in that its sclerotized lining is arranged in eight longitudinal ridges alternating with eight rows of fine hairs.

## Materials

Specimens of *Staphylinus badipes* Lec. were found beneath rocks and logs in damp spots in woods about Ottawa, Ont. on June 20, July 3, and Sept. 6, 1939. They were identified by Mr. W. J. Brown, Division of Systematic Entomology, Department of Agriculture, Ottawa.

Preparatory to making a whole mount of the sclerotized intima of a proventriculus, the digestive tract of a beetle was carefully removed from the body cavity. It was pinned out, under water, on a layer of wax in a Syracuse watch glass, and the proventriculus was slit longitudinally with a pair of fine eye scissors. The proventriculus was cut from the digestive tract and placed in a strong solution of potassium hydroxide, for a few hours, until the layers of muscle and other soft tissues had been dissolved. The hydroxide was then washed out with water, and the sclerotized intima was then washed with a series of alcohols of increasing strength. Then, after washing with xylol, the intima was mounted in Canada balsam.

Serial transverse sections of a proventriculus were made and stained with haemotoxylin and eosin.

## Description

The proventriculus is a spindle-shaped structure and is about 2 mm. long.

The thin, sclerotized intima (I, Figs. 2, 3) is organized into eight longitudinal ridges. In the anterior portion of the proventriculus four of these ridges are larger than those alternating with them (Fig. 2); in the body of the proventriculus the ridges are of equal size (Fig. 3). In the anterior region the crests of the four large ridges are beset with small spines (Fig. 2); in the body of the proventriculus there are long fine hairs that arise from the intima at the sides of the ridges and between the ridges (CH, Figs. 1, 3). These hairs project into the lumen in eight separate clusters that meet at the centre of the lumen and tend to intermingle (Fig. 3). The flattened-out intima of

[1] *Manuscript received January 15, 1947.*

*Contribution from Department of Applied Biology, University of Western Ontario, London, Ont.*

*Part of a thesis presented in accordance with the regulations for the degree of Master of Arts of the University of Western Ontario, London, Ont.*

[2] *Lecturer in Zoology, McMaster University, Hamilton, Ont.*

the proventriculus has the appearance shown in Fig. 1. The rows of hairs are arranged in pairs, and between these pairs of rows are smaller subsidiary rows of shorter hairs.

Beneath the intima is a layer of epithelial cells of irregular shape (EP, Figs. 2, 3, 4) and in the cavities of the longitudinal ridges are strands of longi-

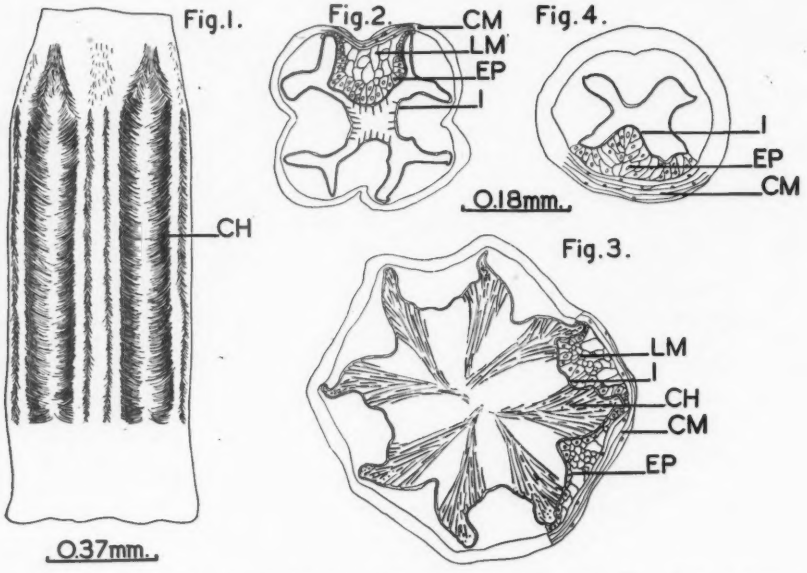



FIG. 1. *Part of the sclerotized intima of the proventriculus showing four of the eight rows of hairs.*

FIG. 2. *Transverse section of the anterior region of the proventriculus.*

FIG. 3. *Transverse section of the middle region of the proventriculus.*

FIG. 4. *Transverse section of the oesophageal valve.*

*CH—coating of sclerotized hairs; CM—circular muscle; EP—epidermal layer; I—sclerotized intima; LM—longitudinal muscle.*

tudinal muscle (LM, Figs. 2, 3). Forming the outer layer of the proventriculus is the circular muscle (CM, Figs. 2, 3, 4). It is two to four strands thick except in the region of the oesophageal valve (Fig. 4) where it is four to six strands thick.

In the region of the oesophageal valve (Fig. 4) the intima is arranged in a few irregular folds, and bears no coating of hairs.

## Discussion

The proventriculus of the rove-beetle, *Creophilus villosus* Grav., is described by Talbot (4). Her description indicates that the proventriculus of *C. villosus* and *S. badipes* are similar. In both species the intima is organized in eight longitudinal ridges and the eight rows of fine hairs project into the lumen.

Heymons and von Lengerken, in a series of papers on the family Silphidae, described the proventriculi of the silphid beetles *Phosphuga atrata* L. (1), *Ablattaria laevigata* F. (2), and *Silpha carinata* L. (3). Their figures indicate that the proventriculus of beetles of this family is similar to that of the Staphylinidae. Eight longitudinal folds are evident, and they are beset with a coating of fine hairs projecting into the lumen.

## Acknowledgment

The writer wishes to thank Professor J. D. Detwiler, University of Western Ontario, for his assistance and interest in this work.

## References

1. HEYMONS, R. and LENGERKEN, H. VON. Studien über die Lebenserscheinungen der Silphini. Z. Morphol. Ökol. Tiere, 9 : 271-312. 1927.
2. HEYMONS, R. and LENGERKEN, H. VON. Studien über die Lebenserscheinungen der Silphini. Z. Morphol. Ökol. Tiere, 24 : 259-287. 1932.
3. HEYMONS, R. and LENGERKEN, H. VON. Studien über die Lebenserscheinungen der Silphini. Z. Morphol. Ökol. Tiere, 25 : 534-548. 1932.
4. TALBOT, M. The structure of the digestive system in *Creophilus villosus*. Ohio J. Sci. 28 : 261-266. 1928.

# ON THE CHEMOTHERAPY OF CAECAL COCCIDIOSIS (*EIMERIA TENELLA*) OF CHICKENS

## III. OBSERVATIONS ON SULPHONAMIDES IN THE INTESTINAL TRACT AND BLOOD[1]

By W. E. Swales[2] and G. H. Benham[3]


## Abstract

Analyses of the contents of the caecal pouches and the small intestine have been made on chickens sacrificed four hours after dosing with sulphonamides. Sulphamerazine was found constantly in the caeca in small amounts even when the caeca were surgically detached from the rest of the intestinal tract, thus showing that the drug is excreted via the caeca to some extent. This property was found to be common also to sulphathiazole, sulphapyridine, and sulphanilamide. The importance of this finding lies in the fact that caecal coccidiosis of chicks is inhibited by sulphamerazine but not by the other three sulphonamides mentioned. It follows that the inhibitory effect is related to drug structure rather than to drug concentration.


The work by Horton-Smith and Taylor (4 and 5), Swales (12 and 13), Hawkins (3), Levine (6–9), Farr and Allen (2), and Ripsom and Herrick (11) on the use of sulphonamides in the control of acute coccidiosis of chickens raises a problem in regard to the mode of action of these drugs in inhibiting the parasite. The drugs that have been shown to have a marked coccidiostatic effect are sulphadiazine (2-sulphanilamidopyrimidine), sulphamerazine (2-sulphanilamido-4-methylpyrimidine), and sulphamezathine or, in the United States, sulphamethazine (2-sulphanilamido-4,6-dimethylpyrimidine). As these drugs are effective in controlling the disease even after haemorrhage from the intestinal tract has begun, it is evident that they inhibit the merozoites and thus prevent or suppress the repetition of schizogony. This effect was suggested for the sulphonamide-pyrimidine drugs by Swales (12) and independently for sulphadiazine by Ripsom and Herrick (11). It is also evident that other sulphonamides, notably sulphaguanidine and sulphathiazole, while not having a curative effect, do prevent acute coccidiosis caused by *Eimeria tenella* when they are given at the time of infection; thus it may be presumed that in the case of these drugs their action in inhibiting the parasite is confined to the sporozoites.

The ability of the sulphonamide-pyrimidine drugs to inhibit a parasite in the caecal pouches of chickens is more peculiar when the fact that these drugs have the characteristic of being rapidly absorbed from the upper digestive tract of animals is considered. Also, the caecal pouches of birds are not

[1] *Manuscript received March 29, 1946.*

*Contribution from the Institute of Parasitology, Macdonald College, McGill University, and the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, with financial assistance from the National Research Council of Canada.*

[2] *Animal Pathologist, Science Service, Dominion Department of Agriculture, Institute of Parasitology, Macdonald College.*

[3] *Research Assistant, Institute of Parasitology, Macdonald College, McGill University. (Now Associate Professor, Department of Biology, Illinois Institute of Technology, Chicago, Ill.)*

readily entered by the contents of the other parts of the intestinal tract and if drugs are to reach the site of *E. tenella* infection via the intestinal tract the therapeutic effect would be very uncertain. Thus it became apparent during the experiments in chemotherapy that sulphamerazine was reaching the caecal pouches via the blood stream. The question arose as to whether the structure or the concentration of the drug was responsible for inhibiting the parasite and in order to elucidate this point a series of observations on the fate of certain sulphonamides in the intestinal tract of the chicken have been made.

## Experimental Methods

The birds used were male Barred Plymouth Rocks, approximately 2½ lb. in weight. In the experiments involving dosing per os each bird was given 75 mgm. of the appropriate drug in a hard gelatine capsule, which, in some cases, included a small amount of gentian violet as a tracer. In experiments involving intravenous medication a 5% aqueous solution of the sodium salt of the drug was injected via the wing veins.

In some experiments the caeca of the birds were ligated close to the ileo-caecal sphincter; this was done, with catgut, under ether anaesthesia and through an incision made through the abdominal wall anteroventral to the cloaca and slightly to the right side. Care was taken to avoid the inclusion of mesenteric blood vessels in the ligature. The drug was administered following recovery from the anaesthetic.

All birds were killed by stunning and bleeding, usually four hours after the drug had been administered. The intestines were removed, the serous surfaces washed free of blood, and the contents expressed by gentle massage. Blood was collected when the birds were killed.

The small intestinal and caecal contents were dried at 110° C., weighed, and ground finely.

Representative samples were taken and extracted with hot *N*/10 sodium hydroxide. The mixture was centrifuged and the supernatant withdrawn. This extraction was repeated three times and the supernatants pooled. This procedure was found to remove completely all the sulphonamides present, whereas neither water nor 95% ethyl alcohol was satisfactory in this respect. Continuous extraction in a Soxhlet extractor, as recommended for tissues by Bratton and Marshall (1), was found to be unnecessary. The combined extracts were made up to a suitable volume, and aliquots were appropriately diluted for sulphonamide determination.

The method used was that of Bratton and Marshall (1), in which diazotization followed by coupling with *N*(1-naphthyl) ethylenediamine dihydrochloride in acid solution produces a red dye. The intensity of this colour was measured in a Klett-Summerson photoelectric colorimeter using green filter No. 54. Blank determinations were made simultaneously by omitting the sodium nitrite from the mixture of reagents.

Determinations on whole blood were carried out as follows: 1 ml. of whole blood was laked in 13 ml. of distilled water, and after three minutes 6 ml. of 15% trichloracetic acid was added with thorough shaking. The precipitate was left for five minutes to settle, after which it was filtered off through a Whatman No. 42 filter paper. The filtrate, representing a 1 in 20 dilution was further diluted if necessary, and the total and free sulphonamide was determined according to the method of Bratton and Marshall. The use of 20% *p*-toluenesulphonic acid instead of 15% trichloracetic acid, as recommended by Morris (10), was found to yield identical results.

The factors for the various sulphonamides used, as determined on pure standard solutions, were as follows:—

Sulphanilamide, 1.00.
Sulphamerazine, 1.39.
Sulphapyridine, 1.46.
Sulphathiazole, 1.47.

## Experimental Observations

Some preliminary observations were first made on two chickens that had been dosed four hours before death with 75 mgm. of sulphamerazine. It was found that a quantity of the drug was present in the caecal pouches and although these tests were not considered quantitatively accurate it was apparent that the amount in the caeca approximated that in the small intestine. These tests were immediately repeated, but this time two birds in which the caeca were ligated were used. Again an appreciable amount of the drug was detected in the caeca, but technical difficulties made a quantitative estimation unreliable.

Following further work by one of us (G.H.B.) on the method of analysis, a series of birds was studied.

Over a period of time seven birds were dosed with 75 mgm. of sulphamerazine per os and were killed four hours after dosing. There was a great variability between the amounts of the drug detected in the intestinal tract; the caeca contained an average of 0.33 mgm. ($\sigma = 0.37$) while the small intestinal contents contained 1.03 mgm. ($\sigma = 0.77$). The complete data are shown in Table I.

Owing to the low values recorded in the above tests five undosed birds were killed and the small intestinal and caecal contents were analysed for blank values. It was thus found that an average of about 27% of the previous estimation of the caecal drug content could be accounted for by the blank values in that part of the intestinal contents (see Table II). However, it was obvious from the analyses of the intestinal contents of the birds dosed with sulphamerazine that some of the drug had entered the caeca via the blood stream, as in the last four of the birds a gentian violet tracer had been used and this had in no case reached the point of junction of the ileum and caeca.

TABLE I

ANALYSIS OF INTESTINAL CONTENTS AND BLOOD FOUR HOURS AFTER DOSING WITH 75 MGM. OF SULPHAMERAZINE PER OS

| Bird No. | Drug in small intestine | | Drug in caeca | | Drug concentration in blood at death (mgm. %)[1] | |
|---|---|---|---|---|---|---|
| | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. contents | Free | Total |
| 2 | 0.48 | 0.51 | 1.17 | 1.04 | Not taken | |
| 7 | 2.10 | 1.00 | 0.04 | 0.18 | 2.56 | 2.86 |
| 9 | 2.20 | 2.00 | 0.06 | 0.27 | 4.50 | 5.10 |
| 10 | 0.38 | 0.40 | 0.18 | 0.29 | 3.80 | 3.90 |
| 11 | 0.105 | 0.32 | 0.08 | 0.13 | — | — |
| 18 | 0.85 | 0.19 | 0.33 | 0.46 | 3.70 | 3.70 |
| 19 | 1.10 | 0.12 | 0.42 | 0.35 | 2.70 | 2.73 |
| Average | 1.03 | 0.65 | 0.33 | 0.39 | 3.45 | 3.66 |
| Standard deviation | 0.77 | 0.61 | 0.37 | 0.28 | 0.73 | 0.85 |

[1] *Mgm. % = mgm. per 100 cc. of blood.*

TABLE II

ANALYSIS OF INTESTINAL CONTENTS OF UNDOSED BIRDS FOR BLANK VALUES

| Bird No. | Small intestine blank values | | Caeca—blank values | |
|---|---|---|---|---|
| | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. |
| 1 *B* | 0.030 | 0.010 | 0.036 | 0.150 |
| 2 *B* | 0.016 | 0.010 | 0.029 | 0.033 |
| 3 *B* | 0.090 | 0.050 | 0.040 | 0.087 |
| 4 *B* | 0.140 | 0.026 | 0.180 | 0.140 |
| 5 *B* | 0.210 | 0.029 | 0.040 | 0.120 |
| Average | 0.097 | 0.025 | 0.065 | 0.106 |
| Standard deviation | 0.070 | 0.015 | 0.058 | 0.042 |

Other sulphonamides were similarly tested. Sulphathiazole in 75 mgm. doses per os, given to similar birds, which were also sacrificed four hours later, likewise was detected in the caeca. On the basis of amount of drug per gram of intestinal contents the results were comparable to those obtained with sulphamerazine (Table III).

Intravenous medication with the sodium salt of sulphamerazine was conducted in order to compare results with those obtained from dosing per os. As shown in Table IV, in five birds the amounts of drug in the caeca were approximately the same as those detected after oral dosing. There was some drug in the contents of the small intestine but this was significantly less than that in the birds dosed per os.

TABLE III

ANALYSES OF INTESTINAL CONTENTS AND BLOOD AFTER COMPARABLE DOSES OF SULPHATHIAZOLE (75 MGM. PER OS—FOUR HOURS)

| Bird No. | Drug in small intestine | | Drug in caeca | | Drug concentration in blood at death (mgm. %) | |
|---|---|---|---|---|---|---|
| | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. contents | Free | Total |
| 2 *C* | 0.650 | 0.96 | 0.17 | 0.21 | — | — |
| 7 *C* | 0.160 | 0.38 | 0.36 | 0.80 | 1.11 | 1.14 |
| 9 *C* | 0.255 | 0.85 | 0.28 | 0.44 | 0.70 | 0.70 |
| 10 *C* | 0.233 | 1.17 | 0.04 | 0.23 | 12.00 | 12.10 |
| 11 *C* | 0.525 | 1.01 | 0.068 | 0.39 | — | — |
| Average | 0.365 | 0.874 | 0.184 | 0.414 | | |
| Standard deviation | 0.189 | 0.242 | 0.126 | 0.212 | | |

TABLE IV

ANALYSES OF INTESTINAL CONTENTS AND BLOOD FOUR HOURS AFTER INTRAVENOUS ADMINISTRATION OF 60 MGM. OF SODIUM SULPHAMERAZINE

| Bird No. | Drug in small intestine | | Drug in caeca | | Drug concentration in blood at death (mgm. %) | |
|---|---|---|---|---|---|---|
| | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. contents | Free | Total |
| 14 | 0.135 | 0.05 | 0.46 | 0.26 | 0.8 | 0.7 |
| 15 | 0.340 | 0.10 | 0.26 | 0.48 | 1.75 | 1.75 |
| 16 | 0.253 | 0.09 | 0.51 | 0.45 | 1.91 | 1.87 |
| 17 | 0.280 | 0.13 | 0.18 | 0.40 | 4.00 | 4.35 |
| 20 | 0.250 | 0.25 | 0.103 | 0.14 | — | — |
| Average | 0.252 | 0.124 | 0.303 | 0.346 | | |
| Standard deviation | 0.069 | 0.068 | 0.160 | 0.300 | | |

Comparable tests were then conducted using sulphanilamide, sulphapyridine, sodium sulphathiazole, and sodium sulphapyridine, the last two being administered intravenously. These limited tests revealed amounts of drug in the caeca and intestine similar to those recorded for sulphamerazine (Table V).

The caeca of three birds were ligated and the birds were later dosed with 75 mgm. of sulphamerazine per os and killed four hours later. The analyses showed that the contents of the small intestine contained an average of 0.35 mgm. (0.29 mgm./gm. contents) of drug and those of the caeca 0.1 mgm. (0.42 mgm./gm. contents). It was evident in these tests that the normal movement of ingesta had been considerably retarded by the manipulation of

TABLE V

CHECK TESTS OF VARIOUS SULPHONAMIDES : ANALYSES OF INTESTINAL CONTENTS

| Bird No. | Drug and dosage | Drug in small intestine | | Drug in caeca | | Drug concentration in blood at death (mgm. % at four hours) | |
|---|---|---|---|---|---|---|---|
| | | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. contents | Free | Total |
| 18 *A* | SA., 75 mgm. per os | 0.440 | 0.114 | 0.097 | 0.187 | 1.11 | 4.11 |
| 18 *B* | SP., 75 mgm. per os | 0.396 | 0.152 | 0.376 | 0.775 | 0.42 | 0.59 |
| 17 *B* | NaST., 60 mgm. intrav. | 0.470 | 0.330 | 0.860 | 0.950 | 0.49 | 0.45 |
| 22 | NaSP., 60 mgm. intrav. | 0.367 | 0.106 | 0.46 | 0.363 | 0.41 | 0.51 |

NOTE: *SA.* = *sulphanilamide.*
*SP.* = *sulphapyridine.*
*NaST.* = *sodium sulphathiazole.*
*NaSP.* = *sodium sulphapyridine.*

the intestines, as the visible tracer was still largely in the crop and gizzard. However, the amount per gram of caecal contents, although small, was 10 times more than the expected blank value.

In most of our tests we set the dose at 75 mgm. and the time between dosing and killing the birds at four hours. In a total of 13 birds, however, using sulphamerazine and sulphathiazole, the doses and times were varied for purposes of comparison. It was found (Table VI) that the amounts detected in the two organs were still very variable and did not follow expected trends.

### Observations on Sulphonamide Concentrations in the Blood

The routine analyses of blood at the time of slaughter of many of the birds revealed great variability in drug concentrations (Tables I, III, IV, V, VI). There was no apparent correlation between the amount of drug in the blood and that in the intestines in these tests. One point of interest is that neither sulphamerazine nor sulphathiazole was conjugated to any extent, but a large proportion of the sulphanilamide and a small proportion of the sulphapyridine were conjugated. These findings of low or absent conjugation of sulphamerazine in chickens is in agreement with the more detailed work of Welch, Mattis, Latven, Benson, and Shiels (14).

### Discussion

The method of collecting the intestinal contents precluded the possibility that our material for analysis contained mucosa or blood in amounts that could be detected in our results. Thus it seems certain that the amounts found in the caecal contents represent excreted drug. It is also apparent

TABLE VI

VARIATIONS IN DOSAGE AND TIMES OF KILLING BIRDS; DRUG LEVELS IN INTESTINAL CONTENTS AND BLOOD

| Bird No. | Drug and dosage | Time, dose to slaughter (hr.) | Drug in small intestine | | Drug in caeca | | Drug concentration in blood at death (mgm. %) | |
|---|---|---|---|---|---|---|---|---|
| | | | Mgm. | Mgm./gm. contents | Mgm. | Mgm./gm. contents | Free | Total |
| 19 | SM. 75 mgm. per os | 4 | 1.10 | 0.12 | 0.42 | 0.35 | 2.70 | 2.73 |
| 19 *A* | SM. 150 " " " | 4 | 0.96 | 0.29 | 0.54 | 0.58 | 5.20 | 5.55 |
| 19 *B* | SM. 300 " " " | 4 | 3.82 | 1.91 | 0.64 | 0.90 | 11.30 | 11.80 |
| 5 | SM. 75 " " " | 1 | 1.34 | 2.20 | 0.28 | 0.28 | 3.08 | 2.86 |
| 4 | SM. 75 " " " | 2 | 4.57 | 1.87 | 0.21 | 0.58 | — | — |
| 6 | SM. 75 " " " | 2 | 1.63 | 1.25 | 0.27 | 0.41 | 9.1 | 8.9 |
| 3 | SM. 75 " " " | 3 | 1.16 | 0.45 | 1.01 | 0.63 | — | — |
| 8 | SM. 75 " " " | 3 | 2.15 | 2.13 | 0.05 | 0.42 | 6.20 | 6.70 |
| 5 *A* | ST. 75 " " " | 1 | 1.38 | 0.97 | 0.25 | 0.25 | 2.02 | 1.93 |
| 4 *A* | ST. 75 " " " | 2 | 0.53 | 0.25 | 0.41 | 0.36 | — | — |
| 6 *A* | ST. 75 " " " | 2 | 0.75 | 0.46 | 0.04 | 0.26 | 1.58 | 1.64 |
| 3 *A* | ST. 75 " " " | 3 | 0.59 | 0.39 | 0.57 | 0.30 | — | — |
| 8 *A* | ST. 75 " " " | 3 | 0.38 | 1.28 | 0.04 | 0.17 | 1.47 | 1.54 |

NOTE: *SM.* = *sulphamerazine.*
*ST.* = *sulphathiazole.*

from the results obtained from intravenous medication that sulphonamides are excreted in the small intestine, as well as in the caeca, but to a somewhat lesser extent.

It is possible that continuous dosing in feed would have resulted in a higher concentration in the caeca over a period of time, but, as the results of our tests had to rely on the fact that the drug could not have entered the caeca from the lumen of the small intestine, the administration of a single dose was the only practical method. However, as this work was designed primarily to find out whether the coccidiostatic action of the sulphonamides was due to drug concentration or drug structure, the experiments showing that sulphonamides other than the coccidiostatic sulphamerazine also appear in the caecal contents appear to have achieved that end. If we had demonstrated that only the sulphonamides that possess the pyrimidine structure possessed the property of being excreted via the caeca, this finding would have suggested that drug concentration rather than structure was responsible for inhibiting some stage of the protozoan parasite, *Eimeria tenella*, in the caeca. It is now apparent that the inhibitory effect on the parasite is probably due to drug structure rather than to any peculiar property of a drug that allows concentration at the site of infection. A full understanding of the coccidiostatic effect of the drugs might rely upon the finding of a specific metabolite used by the merozoites of *E. tenella*, with which the pyrimidine-sulphonamides possibly compete.

## References


1. BRATTON, A. C. and MARSHALL, E. K., JR. A new coupling component for sulfanilamide determination. J. Biol. Chem. 128 : 537-550. 1939.
2. FARR, M. M. and ALLEN, R. W. Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens. J. Am. Vet. Med. Assoc. 100 : 47-51. 1942.
3. HAWKINS, P. A. Sulfamethazine treatment of cecal coccidiosis. Poultry Sci. 12 : 459. 1943.
4. HORTON-SMITH, C. and TAYLOR, E. L. Sulphamethazine and sulphadiazine treatment in caecal coccidiosis of chickens. Vet. Record, 54 : 516. 1942.
5. HORTON-SMITH, C. and TAYLOR, E. L. Saturated solution of sulphamethazine as a substitute for drinking water in the treatment of caecal coccidiosis in chickens. Vet. Record, 55 : 109-110. 1943.
6. LEVINE, P. P. The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. 29 : 309-320. 1939.
7. LEVINE, P. P. The effect of sulfapyridine on experimental avian coccidiosis. J. Parasitol. 26 : 233-235. 1940.
8. LEVINE, P. P. The coccidiostatic effect of sulfaguanidine (sulfanilylguanidine). Cornell Vet. 31 : 107-112. 1941.
9. LEVINE, P. P. Chemotherapy in the control of avian coccidiosis. Proc. Ann. Meeting U.S. Live Stock Sanit. Assoc. 45 : 118-120. 1941.
10. MORRIS, C. J. O. The determination of sulphanilamide and its derivatives. Biochem. J. 35 : 952-959. 1941.
11. RIPSOM, C. A. and HERRICK, C. A. Effects of various sulfa compounds on the protozoan parasite *Eimeria tenella*. J. Parasitol. 31 : 98-108. 1945.
12. SWALES, W. E. On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. Can. J. Research, D, 22 : 131-140. 1944.
13. SWALES, W. E. On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. II. Further studies on the use of drugs in established infections. Can. J. Comp. Med. Vet. Sci. 10 : 3-13. 1946.
14. WELCH, A. D., MATTIS, P. A., LATVEN, A. R., BENSON, W. M., and SHIELS, E. H. Sulfamerizine (2-sulfanilamido-4-methylpyrimidine). I. A comparison of sulfamerizine with sulfadiazine on the basis of absorption, excretion and toxicity. J. Pharmacol. 77 : 357-391. 1943.

# CANADIAN JOURNAL OF RESEARCH

## Notes on the Preparation of Copy

GENERAL:—Manuscripts should be typewritten, double spaced, and the **original and at least one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT:—**An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:*—All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only;** any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil **(do not use typewriting)** and be of such size that they will be legible and not less than one millimetre in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate;** if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—**The author's name, title of paper, and figure number should be written on the back of each illustration.** Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.**

TABLES:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES:—**These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in this Journal. **Titles of papers should not be given in references listed in Sections A, B, E, and F, but must be given in references listed in Sections C and D. All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number; in Sections C and D the author's name and the date of publication may be included with the key number if desired.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual,* published by the Department of Public Printing and Stationery, Ottawa.

## Reprints

Fifty reprints of each paper are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges.